Current Biology Vol 16 No 22
R968

Circadian Rhythms: *Per*turbing a Food-Entrained Clock

When food is scarce, a food-entrainable circadian clock coordinates mammalian activity rhythms with a predictable daily mealtime. Neural and molecular substrates of this circadian function have long eluded localization, but new studies suggest a critical role for a familiar circadian clock gene.

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At a popular waterfront market, not far from here, tourists and city lunchtimers stroll the boardwalk, eating pizza and pastries. Seagulls are quick to snatch a fallen treat, but less obvious are the movements of four-legged scavengers among the rocks below. Why are supposedly nocturnal rats out in the mid-day light? Because that is when the buffet is best. Sounds and smells no doubt provide proximate cues signaling lunch, but several studies dating from the 1920s to the 1990s (reviewed in [1,2]) showed that rats and mice can anticipate a fixed daily meal by entrainment of a circadian oscillator or clock distinct from the clock that generates daily rhythms entrained to light–dark (LD) cycles. A master light-entrainable circadian clock in mammals has been localized to the hypothalamic suprachiasmatic nucleus (SCN), but SCN ablation does not affect food-anticipatory circadian rhythms, so there must be a separate food-entrainable oscillator for behavior elsewhere in the brain or body.

Where is this food-entrainable oscillator? Conventional ablation studies conducted over the past nearly three decades have failed to conclusively identify a critical locus. However, a report by Feillet *et al.* [3] in this issue of *Current Biology* promises to speed the search. Recent years have seen stunning advances in the description of the inner workings of the SCN circadian clock, with the discovery of so-called circadian clock genes (e.g., *Per1, Per2, Cry1, Cry2, Bmal1, Clock, Reverb*α) that form autoregulatory transcription–translation feedback loops thought to drive daily rhythms in individual SCN neurons [4]. These clock genes are also expressed rhythmically in other parts of the brain, and in many peripheral organs and tissues [5], confirming Pittendrigh's insight that multicellular systems are "*literally a population of autonomous oscillators*" [6]. Not surprisingly, meal timing turns out to be the primary entraining stimulus controlling the phase of peripheral circadian oscillators, presumably to ensure optimal synchrony between metabolic processes and the daily rhythm of food intake — be it spontaneous or imposed [5]. An intriguing possibility is that food-anticipatory behavioral rhythms utilize timing cues from peripheral food-entrainable oscillators. However, dissociations between behavioral and peripheral rhythms can be induced quite readily, making this hypothesis unlikely [7].

Assuming that the food-entrainable oscillator that drives food anticipatory behavioral rhythms is located in the brain, one localization strategy is to screen clock gene mutants to identify genes critical for its function and then to map the sites where these genes are expressed in synchrony with mealtime. Conventional lesions or site-specific clock gene knockouts could then be used to confirm the initial localization. Only a few mutants have been screened so far. Mice homozygous for the *Clock* mutation gradually lose circadian rhythmicity in constant dark (DD), but do entrain to LD cycles and retain normal food-anticipatory rhythms [8]. *Cry1/Cry2* double knockouts are immediately arrhythmic in DD, but have residual rhythmicity in LD, and also anticipate daily meals, albeit with reduced intensity [9]. Deletion of *NPAS2*, a paralog of *Clock* widely expressed in the forebrain, may delay the appearance of food anticipatory activity rhythms, but in mice that survive the initial food restriction (significant mortality was observed in knockouts but not wild types) anticipation is essentially normal by 10 days of scheduled feeding [10].

By contrast with these relatively mild food-entrainment phenotypes, Feillet and colleagues [3] now report that food anticipatory rhythms in wheel running, general activity and core body temperature are virtually absent in *Per2*^{brdm1} mutant mice. Given the negative results from prior mutant screens and so many lesion studies, it is comforting to know that the food-entrainable oscillator can apparently be stopped by interfering with a familiar circadian clock gene; it is not a ghost! Nonetheless, more work will be needed to fully interpret the defect. Conceivably, the defect could be in an input pathway necessary for entraining the oscillator or in one or more output pathways that link the oscillator to rhythmic functions. The *Per2*^{brdm1} mice did use their wheels at night and eat as much as wild-type mice during the scheduled daytime meal, so the lack of anticipatory running cannot be easily attributed to impaired locomotion or motivation. While wheel running is normally a reliable assay for food anticipation in genetically and neurologically intact rodents, conventional lesions can eliminate anticipation in one behavioral measure while sparing others [1,11], hence additional behavioral phenotyping is recommended. Operant lever pressing or food-bin approaches are two behaviours that typically exhibit strong food anticipatory rhythms, even in animals that fail to exhibit anticipation in general activity. Additional mealtimes should also be explicitly examined. In food-entrainment studies, the daily meal is usually provided in the middle of the light period, to maximize the signal to noise ratio for detecting food anticipation, as nocturnal rodents with free access to food are quiescent in the day, due to outputs from the SCN pacemaker that suppress activity

and promote sleep [12]. However, rodents also anticipate a nocturnal mealtime, a circadian phase when the SCN pacemaker does not inhibit activity. It is conceivable that normal expression of food anticipatory activity in the daytime requires active suppression of sleep-promoting output from the SCN, and a lesion or knockout may compromise that function. If so, then an animal bearing such a lesion or knockout may express normal food anticipation to a nocturnal meal, or to a meal at any fixed time of day if the SCN is also removed.

If *Per2* is a critical component of the food anticipation mechanism, where are the critical clock cells situated? Feillet *et al.* [3] also measured clock gene rhythms in liver and kidney of *Per2*^{brdm1} mice, and found normal entrainment of these oscillators to a daytime meal, thus strengthening the evidence against a peripheral location of the behavioral food-entrainable oscillator. In the brain, *Per2* is widely expressed, but a recent study has revealed particularly strong expression in the dorsomedial hypothalamic nucleus [13]. Notably, this expression was circadian and evident only in mice that were entrained to a daytime meal. Moreover, the rhythm persisted during two days of total food deprivation, as does the behavioral rhythm, indicating that these rhythms are not simply 'hourglass processes' that must be reset each day by a meal. However, although the dorsomedial hypothalamic nucleus may be a food-entrainable oscillator (pending *in vitro* studies), it cannot be the sole source of timing signals for food-anticipatory behavioral rhythms. While one recent study reported that neurotoxic dorsomedial hypothalamic nucleus lesions strongly attenuated anticipatory rhythms in general cage activity, sleep and body temperature [14], another study published concurrently, using food-bin approaches as the behavioral assay, showed essentially normal food anticipatory rhythms in rats with complete ablation of the dorsomedial hypothalamic nucleus [15]. These results again underscore the importance of comprehensive behavioral phenotyping.

Intriguingly, work reported at a recent conference suggests that the dorsomedial hypothalamic nucleus may participate in the expression of food anticipation to daytime meals by inhibiting sleep promoting signals from the SCN [16]. While at present the evidence is largely circumstantial, such a mechanism could explain why ablation of the dorsomedial hypothalamic nucleus can attenuate at least some food anticipatory rhythms while sparing others, if SCN output during the mid-day normally suppresses general locomotor activity without precluding the expression of a temporally gated preference for a feeding location. Whether this could also explain the *Per2*^{brdm1} food anticipation phenotype is testable. Localization of the food-entrainable oscillator for behavior is not yet resolved, but with the tools at hand and renewed attention, it seems only a matter of time.

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DOI: 10.1016/j.cub.2006.10.020